

CLAIMS

1. A pharmaceutical composition comprising an effective amount of bioactive trans-tetracos-15-enoic acid extracted from a plant *Indigofera tinctoria*, used for the treatment of subjects with hepatic disorders.

5 2. A pharmaceutical composition according to claim 1 wherein said composition along with dehydrocholic acid is used for the enhancement of cholretic activity.

3. A composition according to claim 1 wherein trans-tetracos-15-enoic acid is used singly or in combination with pharmaceutically acceptable additives.

4. A composition according to claim 1 wherein the pharmaceutically acceptable additives are selected from the group consisting of carriers, diluents, solvents, filters 10 lubricants, excipients, binder or stabilizers.

5. A composition according to claim 1 wherein the said composition is used for both preventive and curative properties.

6. A composition according to claim 1 wherein said composition is used systemically, 15 orally or by any clinically/medically accepted methods.

7. A composition according to claim 1 wherein the composition is used to treat hepatic disorders that are clinically, biochemically and histologically similar to that of viral hepatitis, chronic hepatitis, fatty liver, cirrhosis and several vascular lesions of the liver.

20 8. A composition according to claim 1 wherein said composition is used to treat damage induced by hepatotoxins.

9. A composition according to claim 1 wherein the hepatotoxins are selected from the group comprising Galactosamine, Paracetamol, Carbon tetrachloride and alcohol.

10. A composition according to claim 1, wherein the subjects is selected from the group 25 consisting of humans and mammals, preferably humans.

11. A composition according to claim 1 wherein the dosage for the treatment of  $CCl_4$  induced hepatotoxicity in mammals is in the range of 10-100 mg/kg body weight.

12. A composition according to claim 1 wherein the preferred dosage for the treatment of  $CCl_4$  induced hepatotoxicity in mammals is in the range of 50-100 mg/kg body 30 weight.

13. A composition according to claim 1 wherein the hepatoprotective activity in  $CCl_4$  induced hepatotoxic mammals is upto 88%.

14. A composition according to claim 1 wherein the dosage for the treatment of acetaminophen induced hepatotoxicity in mammals is in the range of 10-100 mg/kg body weight.

5 15. A composition according to claim 1 wherein the preferred dosage for the treatment of acetaminophen induced hepatotoxicity in mammals is in the range of 50-100 mg/kg of body weight.

10 16. A composition according to claim 1 wherein the hepatoprotective activity in acetaminophen induced hepatotoxicity in mammals is upto 86%.

17. A composition according to claim 1 wherein the dosage for the treatment of Galactosamine induced hepatotoxicity in mammals is in the range of 10-50mg/kg of body weight.

15 18. A composition according to claim 1 wherein the preferred dosage for the treatment of D-Galactosamine induced hepatotoxicity is in the range of 25-50 mg/kg of body weight.

19. A composition according to claim 1 wherein the hepatoprotective activity in Galactosamine induced hepatotoxicity in mammals is upto 73%.

20 20. A composition according to claim 1 wherein the dosage for the treatment of alcohol induced hepatotoxicity in mammals is in the range of 10-50mg/kg of body weight.

21. A composition according to claim 1 wherein the preferred dosage for the treatment of alcohol induced hepatotoxicity is in the range of 25-50 mg/kg of body weight.

22. A composition according to claim 1 wherein the hepatoprotective activity in alcohol induced hepatotoxicity in mammals is upto 100%.

25 23. A composition according to claim 1 wherein the preferred dosage for hepatic disorders in mammals is about 50-100 mg/kg of body weight.

24. A composition according to claim 1 wherein the preferred dosage for hepatic disorders in human beings is about 10-15 mg/kg of body weight.

25. A process for the isolation of trans-tetracos-15-enoic acid, a bioactive constituent from the plant *Indigofera tinctoria*, said process comprising the steps of:

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5 (a) extracting the powdered plant parts with an aliphatic hydrocarbon solvent at a temperature in the range of 60-80°C for about 20-30 hours;

(b) triturating the extract of step (a) with a ketonic solvent;

(c) separating the solvent soluble portion of step (b) and the residue by using silica gel bed;

10 (d) subjecting the residue of step (c) to column chromatography by eluting with a mixture of organic solvents of increasing polarity to yield a bioactive fraction; and

(e) resolving fraction as obtained in step (e) by high performance liquid chromatography and crystallizing with an alcoholic solvent to obtain the bioactive constituent trans-tetracos-15-enoic acid.

15 26. A process according to claim 24 wherein alternatively, the fraction obtained in step (d) when subjected to semipreparative high performance liquid chromatography, yields bioactive constituent trans-tetracos-15-enoic acid.

20 27. A process according to claim 25 wherein the plant parts used are aerial parts selected from the group consisting of leaves, stem and bark.

28. A process according to claim 25 wherein the hydrocarbon solvent is selected from the group consisting of petroleum ethane, n-hexane, cyclohexane or ligroin and preferably petroleum ether.

25 29. A process according to claim 25 wherein in the ketonic solvent is selected from the group consisting of acetone, ethyl-methyl-ketone, or methyl isobutyl ketone.

30. A process according to claim 25 wherein the solvents used for eluting the column to obtain hepatoprotective fraction are selected from the group consisting of petroleum ether, n-hexane, chloroform, ethyl acetate, a mixture thereof and preferably a mixture of petroleum ether and ethyl acetate.

31. A process according to claim 25 wherein in step (d), the solvent used for performing HPLC is a mixture of Acetonitrile and H<sub>2</sub>O in the ratio of 95:5.

32. A process as according to claim 25 wherein in step (e) the HPLC purified compound is further purified by crystallizing with an alcoholic solvent selected from methanol and isopropanol.

33. A process according to claim 25 wherein the hepatoprotective fraction thus obtained is designated as Indigotone consisting of a mixture of three compounds.
34. A process according to claim 25 wherein the percentage of mixture of three compounds of hepatoprotective fraction is 11-12%, 12-13% and 75-77% respectively.
- 5 35. A process according to claim 25 wherein the hepatoprotective constituent obtained by the process of the present invention is to the extent of 0.09-0.11 % on the basis of dried plant material.
36. A method of treating subjects with liver disorders, the said method comprising administering a pharmaceutically effective dosage of trans-tetracos-15-enoic acid.
- 10 37. A method according to claim 36 wherein trans-tetracos-15-enoic acid is used to treat liver disorders caused by Galactosamine, Paracetamol, Carbon tetrachloride and alcohol.
38. A method according to claim 36 wherein the dosage for the treatment of  $CCl_4$  induced hepatotoxicity in mammals is in the range of 10-100 mg/kg body weight.
- 15 39. A method according to claim 36 wherein the preferred dosage for the treatment of  $CCl_4$  induced hepatotoxicity in mammals is in the range of 50-100 mg/kg body weight.
40. A method according to claim 36 wherein the hepatoprotective activity in  $CCl_4$  induced hepatotoxic mammals is upto 88%.
- 20 41. A method according to claim 36 wherein the dosage for the treatment of acetaminophen induced hepatotoxicity in mammals is in the range of 10-100 mg/kg body weight.
42. A method according to claim 36 wherein the preferred dosage for the treatment of acetaminophen induced hepatotoxicity in mammals is in the range of 50-100 mg/kg of body weight.
- 25 43. A method according to claim 36 wherein the hepatoprotective activity in acetaminophen induced hepatotoxicity in mammals is upto 86%.
44. A method according to claim 36 wherein the dosage for the treatment of Galactosamine induced hepatotoxicity in mammals is in the range of 10-50mg/kg of body weight.
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45. A method according to claim 36 wherein the preferred dosage for the treatment of D-Galactosamine induced hepatotoxicity is in the range of 25-50 mg/kg of body weight.

46. A method according to claim 36 wherein the hepatoprotective activity in Galactosamine induced hepatotoxicity in mammals is upto 73%.

5 47. A method according to claim 36 wherein the dosage for the treatment of alcohol induced hepatotoxicity in mammals is in the range of 10-50mg/kg of body weight.

48. A method according to claim 36 wherein the preferred dosage for the treatment of alcohol induced hepatotoxicity is in the range of 25-50 mg/kg of body weight.

49. A method according to claim 36 wherein the hepatoprotective activity in alcohol induced hepatotoxicity in mammals is upto 100%.

10 50. A method according to claim 36 wherein the dosage for the enhancement of cholretic activity is in the range of 20-50 mg/kg of body weight.

51. A method according to claim 36 wherein the enhanced cholretic activity in mammals is upto 43%.

15 52. A method according to claim 36 wherein trans-tetracos-15-enoic acid is used singly or in combination with pharmaceutically acceptable carriers.

53. A method according to claim 36 wherein the trans-tetracos-15-enoic acid is administered to a subject in combination with pharmaceutically acceptable additives, carriers, diluents, solvents, filters, lubricants, excipients, binder or stabilizers.

20 54. A method according to claim 36 wherein the desired dosage is administered for both preventive and curative properties.

55. A method according to claim 36 wherein trans-tetracos-15-enoic acid is administered systemically, orally or by any clinically/medically accepted methods.

56. A method according to claim 36 wherein the subject is selected from animals, mammals, and preferably humans.

25 57. A method according to claim 36 wherein the preferred dosage for hepatic disorders in mammals is 1about 50-100 mg/kg of body weight.

58. A method according to claim 36 wherein the preferred dosage for hepatic disorders in human beings is about 10-15 mg/kg of body weight.